DIVERSITY OF LOW CHILL PEACHES (*PRUNUS PERSICA*) FROM ASIA, BRAZIL, EUROPE AND THE USA

A Thesis

by

NATALIE ANN ANDERSON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2010

Major Subject: Horticulture

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Approved by:

Chair of Committee,	David H. Byrne
Committee Members,	Stephen King
	J. Creighton Miller, Jr.
	C. Wayne Smith
Head of Department,	Tim D. Davis

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ABSTRACT

Diversity of Low Chill Peaches (*Prunus persica*) from Asia, Brazil, Europe and the USA. (May 2010)

Natalie Ann Anderson, B.S., Texas A&M University

Chair of Advisory Committee: Dr. David H. Byrne

One hundred fifty-five peach (*Prunus persica*) cultivars, from Asia, Brazil, Europe, and the USA, were examined using eleven Simple Sequence Repeats (SSRs) to study the genetic relationships among low chill as compared to high chill peach germplasm. Data was analyzed by NTSYSpc to form a similarity matrix using Nei and Li's Dice similarity coefficient. This similarity matrix was then subjected to a cluster analysis and a dendrogram was constructed using the UPGMA (Unweighted Pair-Group Method, Arithmetic Mean) method. A wide range of diversity was detected, from 0.33 coefficient of similarity amongst the Thai peaches to 0.97 between two Brazilian peaches. The most distant clusters were the low chill peaches from Thailand and Taiwan and the local cultivars (both fruit and ornamental types) from China. Among the improved germplasm, there were distinct clusters for the Chinese/Japanese cultivars, three clusters for the Brazilian cultivars and one for the cultivars from the USA and Europe. The Brazilian materials clustered according to breeding programs in São Paulo and Pelotas reflecting the different sets of local cultivars used in the breeding efforts. The largest group investigated was the European/USA peaches. This group subdivided

into three distinct clusters, with a general clustering of the low chill germplasm. The low chill accessions from Asia were genetically distant from the improved low chill peaches from the USA or Brazil. The low chill peaches from the Americas were more closely related to the high chill peaches developed in the USA and China/Japan due to the introgression of this germplasm into a low chill background.

DEDICATION

To my parents, for all of their support and love through the years.

ACKNOWLEDGEMENTS

I wish to thank the members of my committee for their support and patience. I especially appreciate Dr. David Byrne's help and guidance throughout the years.

My heartfelt thanks go to the members of the Rose and Peach Breeding Program, including all graduate students, student workers and visiting scientists whose help I will never be able to repay!

I also wish to thank a special man in my life, for encouraging me when I had given up, holding me close when I needed a shoulder to cry on and for celebrating all of the 'small' milestones with me.

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INTRODUCTION AND LITERATURE REVIEW

For hundreds of years, the origin of the peach has been incorrectly reported and in turn this information was passed down erroneously from generation to generation. Even the botanical name of peach, *Prunus persica* (L.) Batsch, conveys that its origins were in ancient Persia (modern day Afghanistan, Iran, Iraq, Pakistan, Syria, Turkey, Turkmenistan, Uzbekistan, the southern regions of Kazakhstan and the northern portions of Egypt). Only in the past 150 years has the opinion emerged that the true origin of the peach is China, not ancient Persia as first believed (Bunyard, 1938; Faust and Timon, 1995; Hedrick et al., 1917).

Evidence for the belief China is the country of origin of the peach is found in many sources, the strongest of those being in both the archeological and written records. Huang et al. (2008), citing an earlier reference, reports that wild peach pits dating between 7000 and 6000 BC were discovered at an archeological dig of a Neolithic site found in Zhejiang Province, China. Furthermore, Needham and Wang (2008) reported that several ancient Neolithic sites located in the same province of China were reported to have peach pits dating to approximately 5000 BC.

Currently, the earliest written record for peach is found in the Shi Jing, a book of

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This thesis follows the style of the American Society of Horticultural Sciences Journal.

songs and poems, compiled during the Zhou Dynasty which ruled during 1027-771 BC (Faust and Timon, 1995). The author of this book has been lost to history, however this earliest mention of peach reconfirms the center of origin was most likely China.

In 1984, Zai-long reported that the method of transport and the year in which the peach was exported out of China is unknown. The most probable method of export was by seed through caravans that traveled from China westward on the land routes termed the Silk Road (Faust and Timon, 1995; Hedrick et al., 1917). The Silk Road was actually several extensive land (and later sea) routes that connected China to the countries located to the west (Wood, 2002).

Although a date has not been found to associate the arrival of the peach into Persia, Monet and Bassi speculated that it arrived in the 2nd or 1st century BC (Monet and Bassi, 2008; Faust and Timon, 1995). This assumption is further backed by Ghirshman (1954) who spoke of a formal exchange that occurred around 115 BC of plants such as cucumber, jasmine, onion and saffron from Persia, and apricots and peaches from China (Abivardi, 2001).

The land route of the Silk Road terminated along the west coast of Persia (modern day Syria). With the Roman occupation of this costal region, peaches were carried onward throughout the Roman Empire (Faust and Timon, 1995).

The introduction of the peach into the New World had several paths. One of the earliest was by the Spanish Conquistadors who arrived in Central America in the early 16th century. Hedrick et al. (1917) reports that in less than 50 years after Hernán Cortes conquered the Aztec Empire, peaches were commonly grown throughout the region.

From Central America, the peach spread northward to the southwestern regions of North America (Faust and Timon, 1995). A second separate introduction occurred from Spain in 1565 when the Spaniards founded the city of St. Augustine located along the Florida coastline (Hedrick, 1917).

Another introduction of peaches into the New World occurred with the English settlement of Jamestown, Virginia in 1607 (Christ, n.d.). Apparently, peaches grew so well in this region that when Captain John Smith visited Jamestown in 1629 he wrote of "peaches in abundance" (Hedrick et al., 1917).

Peaches were imported directly from China into the United States of America (USA). A group of peaches in 1850 that were initially referred to as 'Chinese Cling' and 'North China' were shipped to the Delaware Experimental Station (Faust and Timon, 1995). It was unspecified if these cultivars were shipped as trees or seeds, but the most probable way was as seeds.

The introduction of peaches into other parts of the world, such as South America, is not well documented. In 1532 Martin Afonso de Souza brought peach plants to Sao Vicente, which is modern day São Paulo, Brazil (Raseira et al., 2008). The only other mention of peaches in South America that could be found was by Capparelli et al. (2005) who uses archaeobotanical and ethnohistorical evidence to trace the first introduction of peach (mid 1500s) into northwest Argentina from three possible sources: the Atlantic Ocean, from Peru and from Chile.

Botanical

Currently, the botanical classification for peach is *Prunus persica* (L.) Batsch (Faust and Timon, 1995). It is a diploid (2n = 16), dicotyledon that is self fertile (Baranek et al., 2006). Due to the self fertile nature of peach, classification within the genus is sometimes controversial, however there is wide acceptance for the order of Rosales, family of Rosaceae and subfamily Prunoideae (Baranek et al., 2006; Smith, 1977; Zhebentyayeva et al., 2008). The Prunoideae subfamily is characterized by plants that produce a fruit type called a drupe. Botanically speaking, drupes are a fruit that have a soft, edible exocarp/mesocarp that surround a hard, lignified endocarp which in the case of *Prunus persica* is referred to as the stone or pit (Abbott et al., 2007).

The reported number of *Prunus* species varies from as small as 77 (Watkins, 1976) up to 200 (Bortiri et al., 2001). However, over 4,300 species, varieties and cultivars exist within the *Prunus* genus which includes peaches, nectarines, plums, apricots, cherries, almonds and mume (GBIF Data Portal, www.gbif.net, January 2009).

Peaches are grown in both the Northern and Southern Hemispheres of the world and absent only from the continent of Antarctica and the landmass of Greenland (GBIF Data Portal, www.gbif.net, January 2009). Even though peaches have adapted to the

various climates located throughout the world, the bulk of peach production occurs in the temperate zones which are located between the Tropic of Cancer and the Artic Circle in the Northern Hemisphere and the Tropic of Capricorn and the Antarctic Circle in the Southern Hemisphere (Westwood, 1993).

Economic Importance

Prunus persica (L.) Batsch includes both peaches and nectarines of which 17.5 million tons were produced in 2007 (FAOStat, http://faostat.fao.org, 26 February 2009). In the production of temperate tree fruits, *Prunus persica* was surpassed only by apples at 64 million tons and pears at 20 million tons (FAOStat, http://faostat.fao.org, 26 February 2009). In the ten year period from 1997 to 2007 production has risen from 11.4 million tons to 17.5 million tons (Figure 1) (FAOStat, http://faostat.fao.org, 26 February 2009).

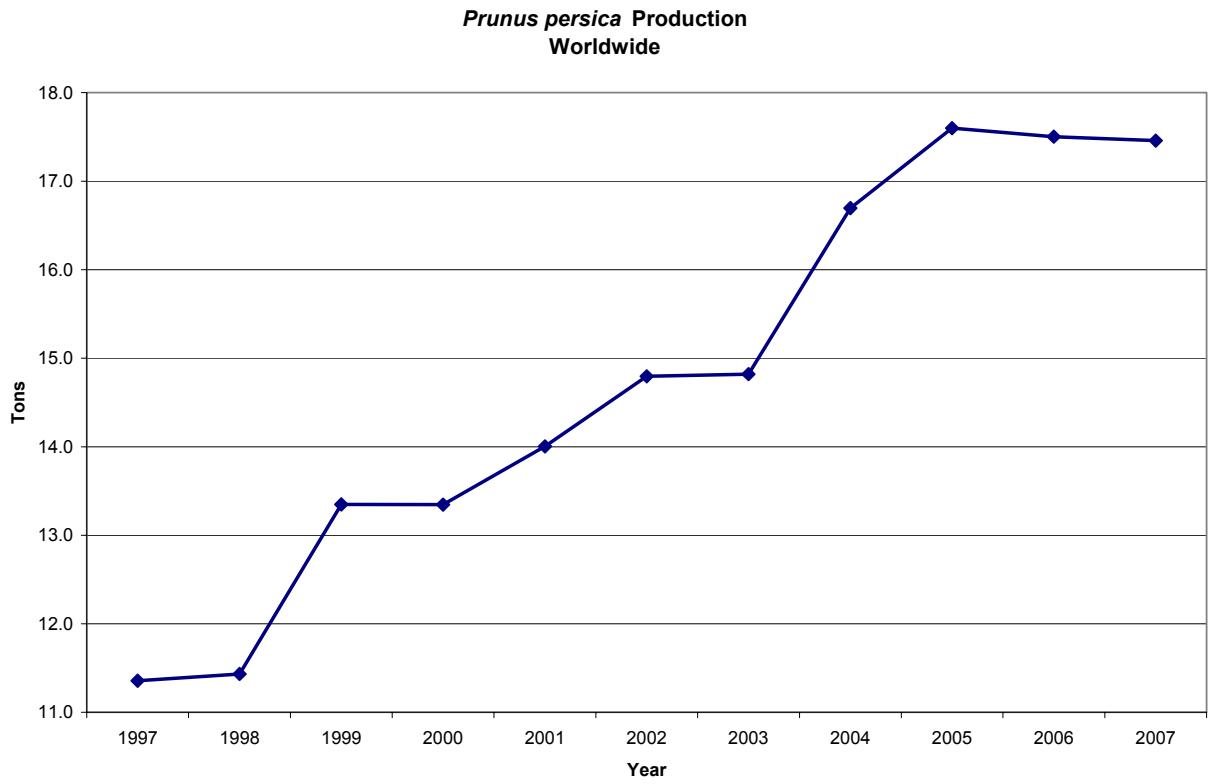


Figure 1. *Prunus persica* production worldwide. Tons of peaches and nectarines (*Prunus persica* L. Batsch) produced from 1997 through 2007 (FAOStat, http://faostat.fao.org, 26 February 2009).

Molecular Techniques

Before molecular techniques were created, visual characteristics were used to distinguish between various peach cultivars/species. Unfortunately, morphological characteristics such as flower, fruit and leaf shape are influenced by the environment (Bernhard 1991; Tavaud et al., 2004). Another drawback of morphological characteristics is the limited number of traits available for study (Kuleung et al., 2006).

A protein electrophoresis technique, developed in 1957 by Hunter and Markert, provided a new source of heritable traits for scientists to study (Parker et al., 1998). This technique, referred to as isozymes, was the first extensively used molecular marker technique. In *Prunus* the isozyme technique has been used to identify cultivars and hybrids (Byrne and Littleton, 1988, Byrne and Littleton, 1989, Carter and Brock, 1980; Parfitt et al., 1985; Torres, 1983), estimate levels of genetic variability (Arulsekar et al., 1986; Byrne, 1990; Frascaria et al., 1993) and construct genetic linkage maps (Clarke et al., 2009; Dirlewanger et al., 1998). Although this technique has the advantage of being relatively inexpensive and having codominant markers, it has slowly fallen out of favor due to its limitation of the relatively small number of loci it is able to detect (de Vicente et al., 1998).

The next evolution of molecular markers came about in 1985 with the development of Restriction Fragment Length Polymorphisms (RFLPs) by Jeffreys et al. In this technique, DNA is isolated from the plant and digested with restriction enzymes. After digestion of the DNA, the fragments are separated on a gel and their band profiles are compared.

The RFLP technique has been used extensively in *Prunus* to produce genetic linkage maps (Dirlewanger et al., 1998; Dettori et al., 2001; Eldredge et al., 1992; Foolad et al., 1995; Joobeur et al., 1998; Quarta et al., 1998; Rajapakse et al., 1995; Viruel et al., 1995; Wang et al., 1998) and to a lesser extent for *Prunus* diversity studies (de Vicente et al., 1998; Nybom et al., 1990; Quarta et al., 2001). This may be due to the disadvantage of RFLP being a non-PCR (Polymerase Chain Reaction) type marker, therefore requiring large amounts of DNA for analysis. This particular type of technique is also quite costly and time consuming, reducing its utility for diversity studies as compared to other techniques. However, RFLPs have the advantage of producing codominant markers which are highly reproducible (McDonald, 1997).

With the advent of PCR, several new molecular markers became available. One of these markers, called Randomly Amplified Polymorphic DNAs (RAPDs) has been used widely in a variety of applications. Genetic diversity has been studied by several in *Prunus* for both fruit producing tree types and rootstock cultivars (Casas et al., 1999; Lu et al., 1996; Warburton and Bliss, 1996). Numerous genetic linkage maps have also been produced using RAPD markers in *Prunus* (Dettori et al., 2001; Rajapakse et al., 1995), *Rosa* (Debener and Mattiesch, 1999; Rajapakse et al., 2001), maize (Beaumont et al., 1996) and cotton (Lin et al., 2005) to name a few.

The extent of RAPD marker utilization is due to its relative ease of use, basis on the PCR technique, abundant polymorphism and low costs (Warburton and Bliss, 1996). The drawbacks of using RAPD markers are the dominant nature of the banding patterns and the low transferability between labs.

The next advance was the Amplified Fragment Length Polymorphisms (AFLPs) which is the combination of the RFLP method with PCR techniques. This cost effective method is capable of producing a large number of complex banding patterns while maintaining highly reproducible results (Weising, et al. 2005). The method is so robust it has been used for a large number of techniques including taxonomy, diversity, pedigree analysis, population genetic studies, genetic linkage mapping, physical mapping and the identification of cultivars, hybrids and clones in a wide range of plant species (Debener and Mattiesch, 1999; Geuna, et al. 2003; Weising, et al. 2005; Xu et al., 2006). AFLP markers do have a few drawbacks, such as a dominant marker type, clustering of markers around certain regions of the chromosome and the huge amount of information produced (Debener and Mattiesch, 1999; Weising, et al. 2005)

Simple sequence repeats (SSRs), also known as microsatellites, are tandem repeated units of one to six nucleotides. This technique was developed by Lit and Luly in 1989 while searching for an alternative method for studying polymorphisms in the human genome. Although the initial set-up for the SSR technique can be rather expensive, it has many desirable characteristics including their abundance and distribution throughout the genome, a high degree of polymorphism, its codominant nature, a relatively large number of alleles per loci, it is PCR based, it is highly reproducible within and between labs, and the markers have good transportability across species within the same genus (Ellis and Burke, 2007; Sosinski et al., 2000; Zhebentyayeva et al., 2003).

Numerous articles have been published utilizing SSR markers in *Prunus* over genetic linkage mapping (Aranzana et al., 2003; Dettori et al., 2001; Joobeur et al., 2000; Sanchez-Perez et al., 2006), primer development (Downey and Iezzoni, 2000; Dirlewanger et al., 2002; Gil-Ariza et al., 2006; Mnejja et al., 2004), parentage analysis (Yamamoto et al., 2003a; Yamamoto et al., 2003b) and genetic diversity (Baránek et al., 2006; Maghuly et al., 2005; Marchese et al., 2005;2006; Wünsch et al., 2006; Zeinalabedini et al., 2008). Investigations in other plant species have utilized the SSR technique as well, including potato (*Solanum tuberosum* L.) (McGregor et al., 2000), olives (*Olea europaea* L.) (La Rosa et al., 2003) and roses (Yan et al., 2005).

Diversity

Genetic diversity is essential for any breeding program. Not only is it necessary for the incorporation of improved horticultural characteristics such as flavor and increased production, it is also needed for the integration of disease and pest resistance. Given the economic importance of *Prunus*, several diversity studies have been performed on this crop within the United States of America (Byrne, 1989; Byrne, 1990; Scorza et al., 1985; Scorza et al., 1988) and throughout the world (Badenes, 1998; Byrne and Raseira, 2006; Casas, et al., 1999; Lansari et al., 1994; Ma et al., 2006; Marchese et al., 2006; Quarta et al., 2001; Warburton and Bliss, 1996; Yoon et al., 2006), however the majority of these only look at diversity of the European/USA germplasm.

Pedigree Analysis

Pedigree studies of peaches found in the United States of America have focused on both low and high chill adaptation regions. In 1985, Scorza et al. studied 30 freestone

peach cultivars developed in the eastern USA (high chill) and found that the mean inbreeding coefficients were relatively high: 0.26 for Case I (outcrossing assumed) and 0.35 for Case II (selfing assumed). These relatively high coefficients are due to the self fertile nature of peaches and the repeated use of cultivars derived from 'Chinese Cling' and a few other key cultivars such as 'Elberta', 'J. H. Hale' and 'St. John' (Scorza et al., 1985). Subsequent studies showed less inbreeding among the high chill Chinese commercial cultivars (Ma et al., 2006), and the low chill cultivars developed in Florida (Scorza et al., 1988) and Brazil (Byrne and Raseira, 2006). The lower inbreeding coefficients of the Chinese, Florida, and Brazilian peach cultivars are due to recent incorporation of exotic germplasm as well as the use of traditional selections as commercial cultivars (Ma et al., 2006; Scorza et al., 1988; Byrne and Raseira, 2006).

Founding Clone Analysis

During the development of low chill, melting flesh, fresh market peach germplasm in the USA, the low chill trait (less than 250 chill units) was derived mainly from the south Chinese genotypes of 'Hawaiian', 'Jewel', 'Lukens Honey', 'Okinawa', and 'Peento' (Byrne and Bacon, 1999). As these sources of low chill had generally poor commercial characteristics, they were crossed with high chill cultivars with higher commercial quality. Consequently, a small number of high chill cultivars ('J. H. Hale', 'July Elberta', 'Rio Oso Gem' and 'St. John') have contributed to the low chill germplasm as well (Byrne and Bacon, 1999).

The commercial low chill breeding programs in Brazil (São Paulo and Pelotas) and Mexico (Chapingo and Queretaro) have exploited local cultivars that had been

propagated by seed and selected over many generations for their adaptation and commercial characteristics (Byrne et al., 2000). In the breeding of melting flesh peaches for the fresh market, the Pelotas program used the local cultivars '15 de Novembre', 'Admirável', 'Delicioso' and 'Precoce Rosado', whereas the program in São Paulo used 'Rei da Conserva', 'Perola de Itaquera' and 'Taichi' (Byrne and Bacon, 1999). It has been estimated that these local selections have contributed from about ⅓ to ½ of the genetic background of the current modern Brazilian cultivars (Byrne and Bacon, 1999). Additionally, the low chill breeding programs of Brazil and Mexico have exchanged germplasm with the low chill breeding programs of the USA, and subsequently incorporated the USA low chill founding clones into their germplasm.

In the development of non-melting peaches for both the processing and fresh markets located in low chill zones, three programs have been active. The longest running program is the non-melting program with EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) in Pelotas, Brazil. Its work has used four local cultivars in breeding: 'Aldrighi', 'Ambrósio Perret', 'Abóbora', and 'Intermediario' (Byrne and Bacon, 1999). In the 1980s, breeding efforts in Mexico were initiated in Queretaro and Chapingo with the aim of developing non-melting peaches for the fresh market. Both of these programs used local selections and the Brazilian cultivar 'Diamante' extensively in their breeding (Byrne et al., 2000).

Molecular Based Diversity

Through the years, isozyme reports have consistently indicated that low heterozygosity (0.02-0.03) exist within the US/European cultivars studied (Byrne, 1999;

Durham, et al., 1987). Several of these studies show that non-melting peach germplasm is more diverse than USA/European melting peaches and nectarines (Ibanez et al., 1993; Messeguer et al., 1987). However, one report using more isozymes than previous studies, and an acrylamide gel system which gives better resolution than the standard starch gel system, found a greater amount of polymorphism among the cultivars than had previously been reported (Gašić et al., 2000). This study also indicated that the North American and Italian cultivars clustered separately from the majority of the Japanese and Chinese cultivars indicating that these groups were genetically distinct.

In 1996, Warburton and Bliss used RAPD markers to study 136 cultivars from 20 different countries and found that the US, European and Latin American peaches grouped into 3 of 12 clusters which exhibited the least amount of diversity. An interesting conclusion from this study was that Florida cultivars formed their own cluster indicating that exotic germplasm had been utilized for low chill adaptability (Warburton and Bliss, 1996). The other nine clusters comprised a range of cultivars from Asia including one cluster with the low chill cultivar 'Okinawa' (Warburton and Bliss, 1996). This cultivar is one of the key sources of the low chill trait used by the Florida breeding program in its development of low chill cultivars (Byrne et al., 2000). Furthermore, Badenes et al. (1998) comparing local Spanish peach populations and North American cultivars with RAPD markers found that the local Spanish non-melting germplasm was distinct from the North American melting flesh cultivars.

Limited work has been done with AFLPs (see Table 1). Promchot et al., found that all of the 23 local Thai accessions studied were closely related to each other and to

the 3 low chill US cultivars included in this study (2005). However, when all of these accessions/cultivars were compared with one northern China peach it was found that they were distantly related (Promchot et al., 2005). Two separate studies using a combination of AFLP markers with either RAPDs or SSRs, found that of the cultivars tested, all (but one) could be distinguished from each other, however there was no clear separation between the USA and European cultivars (Aranzana et al., 2001; Dirlewanger and Duha, 1998).

Although most of the peach diversity studies utilizing SSR markers have focused on a relatively narrow range of germplasm (Table 1), one study used a wide selection of germplasm (white and yellow flesh, melting and non-melting flesh, local and improved cultivars and ornamentals) from Asia (China, Japan, and Korea) and North America (Yoon et al., 2006). This study established that the ornamental and dwarf peach germplasm was distantly related to the types used for fruit production. Among those for fruit production, two large clusters were detected: one containing two subgroups with local cultivars from the northern and western regions of China; the other contained four subgroups which included cultivars from southern China, Japan, Chinese flat and North American yellow types (Yoon et al., 2006).

Table 1. Summarization of molecular diversity studies.

Marker type (No. used)	Germplasm assayed (No. assayed)	Conclusions	Reference
Isozyme (6)	Peach (12) - 9 USA, 1 USA rootstock and 2 Japan	Six loci out of 15 were polymorphic.	Agarwal et al., 2001
Isozyme (6)	Peach (290), Almonds (87)	In peaches, only 1 out of 12 isozymes were polymorphic.	Arulsekar et al., 1986
Isozyme (12)	Peach (86), Almonds (4), Plums (51), Apricot (79)	Heterozygosity for peaches is very low when compared to almonds, apricot and plums	Byrne, 1990
Isozyme (32)	Peach (33) - 10 USA, 27 Italy, 4 China and 6 Japan	Greater isozyme activity than previously reported. China/Japan cultivars separated from European cultivars.	Gašić et al., 2000
Isozyme (3)	Peach (26) - USA, Spain and Argentina	Low variability, with nectarines having the lowest.	Ibanez et al., 1993
Isozyme (14)	Peach (81) - 52 US, 20 Spain, 3 France, 1 New Zealand, 4 South Africa, 1 Italy	Freestone cultivars less variable than clingstone	Messeguer et al., 1987
RAPD (40)	Peach (22) - 11 Spanish clones and 11 North American cultivars	Dendrogram clustered around geographic origins	Badenes et al., 1998
RAPD (94)	Peach (136) – 91 USA, 9 European, 3 South America, 2 Mexico, 2 Pakistan, 18 China, 2 Japan, 2 India, 1 South Africa, 1 Korea, 4 Unknown + 1 Almond	Most European, Latin America and US cultivars grouped into 3 clusters. Least amount of diversity found in cluster which contains Western, non-melting, clingstone peaches. Other nine clusters contain peaches from other countries.	Warburton and Bliss, 1996

Table 1. Continued.

Marker type (No. used)	Germplasm assayed (No. assayed)	Conclusions	Reference
RAPD (35)	Peach (29)	Dendrogram formed 7 groups: Brazil, US, S China, Central China/Japan, Dwarf, Li Ho Wan Tao group and Chi Chen Tao group. Japan peach may originate from central China	Wen and Chieh, 2003
RAPDs (79) + RFLPs (28)	RAPD: Peach (61), Almond (1), Peach x Almond (4) RFLPs: Peaches (39)	Cluster analysis showed 80-100% similarity for both RAPD and RFLP results.	Quarta et al., 2001
SSR (21) + RAPD (40)	Peaches (9) – European/US cultivars	SSR: Similarity coefficient 0.72 for peaches. RAPD: Similarity coefficient 0.96 for peaches.	Baránek et al., 2006
RAPD (100) + AFLP (14)	Peaches (63) – European/USA cultivars	Distinguish by cultivars.	Dirlewanger and Duha, 1998
SSR (7) + AFLP (40)	Peach/Nectarine (100) - European/USA cultivars	When combining techniques, all but one could be distinguished.	Aranzana et al., 2001
AFLP (10)	Peach (27) – 23 Thailand, 3 low chilling & 1 China	All Thai and low chilling peaches clustered together, Chinese peach most distantly related.	Promchot et al., 2005
AFLP (9)	Peach (86) – Chinese Local accessions	S and NW local cultivars clustered separately from N and wild cultivars.	Wang et al., 2008
AFLP (16)	Peach (23) – Japanese commercial & traditional accessions	All cultivars could be identified. Generally, commercial peaches are distantly related to traditional accessions.	Xu et al., 2006

Table 1. Continued.

Marker type (No. used)	Germplasm assayed (No. assayed)	Conclusions	Reference
SSR (36)	Peach (25) – European/USA cultivars	Generally nectarines cluster separately from peaches. Melting and non-melting separate clusters.	Aranzana et al., 2002
SSR (16)	Peach (212) – European/USA cultivars	Nectarine, peach and non-melting flesh clustered separately. Non-melting more variability than rest	Aranzana et al., 2003
SSR (13)	Peach (36)	Nectarines & fresh market peaches clustered separately from canning peaches	Bianchi et al, 2004
SSR (20)	Peach (29) – 19 Miraflores clones & 10 traditional cultivars	Clustered into 2 main groups. One had most Miraflores clones plus 3 cultivars, other group had rest of cultivars studied.	Bouhadida et al., 2007
SSR (34)	Peach (56) – 25 USA, 13 China & 18 Japan	Of three groups studied, genetic diversity was lowest in USA	Chen et al., 2007
SSR (7)	Peach (32) – Chinese local cultivars and improved cultivars	Clustered into 2 distinct groups, local cultivars and cultivars	Cheng and Huang, 2008
SSR (41)	Peach (27) – European/USA cultivars + Cherries	'Nemared' and 'Desertgold' each formed individual clusters, rest grouped together	Dirlewanger et al., 2002
SSR (22)	Peach (51) – 35 China, 7 Japan & 9 USA	Genetic diversity within groups ranked (high to low): Sweet peach, crisp peach, flat peach, nectarine, honey peach, yellow fleshed peach	Li et al., 2008
SSR (15)	Peach (49) – Sicilian & US	Sicilian clustered separately from US cultivars. USA cultivars sub-clustered into CA/Georgia, nectarines, Florida and mix	Marchese et al., 2005

Table 1. Continued.

Marker type (No. used)	Germplasm assayed (No. assayed)	Conclusions	Reference
SSR (15)	Peach (23) – Sicilian	Most indigenous Sicilian peaches clustered separately from USA peaches	Marchese et al., 2006
SSR (18)	Peach (20) + Processing Peach (15) + Rootstock (10) + Almonds (30) + 1 wild ea: P. davidiana, P. webbii	Generally, clingstone processing peaches clustered separately from freestone cultivars.	Martinez-Gomez et al., 2003
SSR (7&10)	Peach (117) – European/US cultivars	Peaches and nectarines cluster separately. Clingstone/melting clusters separately from freestone/non-melting and yellow separately from white	Rojas et al., 2008
SSR (10)	Peach (85) – Local Spanish	Flat and white fleshed separated from yellow non-melting local	Wünsch et al., 2006
SSR (17)	Peach (16) – Japanese	Distinguished among cultivars; useful for parental analysis	Yamamoto et al., 2003
SSR (33)	Peach (96) – China, Japan, North America & South Korea	Clustered into 6 groups reflecting 'ecogeographic origin'	Yoon et al., 2006
RAMP (10)	Peach (41) – Japan, China, USA, Brazil	Cluster analysis groups Japan/China, USA, nectarines, flat peaches separately	Cheng, 2001

The general distinctness between southern and northern/northwestern Chinese peach germplasm is supported by other studies (Ge et al., 2009; Wang et al., 2008) as is the distinctness of fresh market germplasm and ornamental/dwarf germplasm (Wen and Chieh, 2003; Wang et al., 2008). Additionally, the close relationship among southern Chinese, improved Chinese, and Japanese germplasm has also been noted by Gašić et al. (2000), Chen et al., (2007) and Xu et al. (2006). These groups are closely related to North American and European improved cultivars, due in part to the extensive use of 'Chinese Cling' ('Shanghai Suimitsuto') and its relatives in the breeding of these groups of cultivars (Scorza et al., 1985; Yamamoto et al., 2003a).

Among the American and European germplasm, several subgroups appear indicating a clustering of high chill melting peach, melting nectarine, and non-melting peach cultivars (Aranzana et al., 2003; Messeguer et al., 1987; Warburton and Bliss, 1996) with these generally being distinct from local unimproved cultivars found in Spain (Bouhadida et al., 2007) and Sicily (Marchese et al., 2006). Up to now, little work has been done to evaluate the genetic diversity of low chill germplasm in the context of peach germplasm as a whole. However, there is evidence that the low chill USA cultivars are distinct from the high chill germplasm reflecting a recent introduction of exotic germplasm (Marchese et al., 2005; Warburton and Bliss, 1996; Byrne et al., 2000). Furthermore, studies indicate that local and improved low chill germplasm from Asia and Brazil is also distinct from American or Chinese high chill peach germplasm (Warburton and Bliss, 1996; Wen and Chieh, 2003; Promchot et al., 2005).

The objective of this study is to assess the diversity and the relationships among the low chill peach germplasm from Brazil, the USA, and Southeast Asia compared to high chill germplasm from China, Europe and the USA using SSR markers.

MATERIALS AND METHODS

Plant Material

DNA was extracted from a total of 155 peach cultivars/genotypes (Table 2). Of these, 79 were collected from various locations across the United States of America, 6 were collected from Thailand by Unaroj Boonprakob (Department of Horticulture, Kasetsart University, Nakhonpathom, Thailand), 61 were collected from Brazil by Maria Do Carmo Bassols Raseira and Caroline Castro (Clima Temperado, EMBRAPA, Pelotas, RS, Brazil) and 9 were collected from China by Lirong Wang (Zhengzhou Fruit Tree Research Institute, Chinese Academy of Agricultural Sciences, Zhengzhou, Henan, China) (Table 2).

Table 2. List of selections, cultivars, parents and traits.

Genotype name	Geographic source	Genetic origin	Female parent	Male parent	Fruit traits[z]
81-11-58	California, USA	China	Zhaohui	(Fenghuayulu × Wasasuimitsu)	PWMC
Abóbora	Brazil	Brazil	Unknown	Unknown	PYNmC
Aldrighi	Brazil	Brazil	Unknown	Unknown	PYNmC
Arctic Star	Texas, USA	USA	White Nectarine	May Glo	NWMC
Arlequim	Brazil	Brazil	Lake City	Toschina	PWMF
Atenas	Brazil	Brazil	Jade	Unknown	PYNmC
August Prince	Georgia, USA	USA	Sunprince	BY92P2710	PYMF
Autmunprince	Georgia, USA	USA	O'Henry	BY79P670	PYMF
Babcock	California, USA	USA	(Strawberry x Peen-To)	Unknown	PWMF
Baronesa	Brazil	Brazil	[(Hawaiian x Southland) x Unknown] x Unknown	Unknown	PYMS
Blancona	Brazil	Bolivia	Unknown	Unknown	PWMF
Bounty	Texas, USA	USA	B60324	B64237	PYMF
BR1	Brazil	Brazil	Delicioso	Panamint	PWMC
BR6	Brazil	Brazil	Ambrósio Perret	Tapes	PYNmC
Bruna	Brazil	Brazil	NJ238	[(Candoka x Flaming Gold) x NJN14] x Unknown	NYMS

Table 2. Continued.

Cultivar Name	Geographic Source of Sample	Genetic Origin	Female Parent	Male Parent	Fruit Traits[z]
BY03-3107s	Georgia, USA	USA	BY94P3638	BY90P2676	PRMC
BY90P2676	Georgia, USA	USA	BY87P1368	Unknown	PRM-
Cai	Brazil	Brazil	Lake City	Delicioso	PWMC
Cardeal	Brazil	Brazil	FV338-90	Unknown	PYMS
Chimarrita	Brazil	Brazil	Babcock	Flordabelle	PWMF
Chiripa	Brazil	Brazil	Delicioso	Nectared 5	PWMF
Chula	Brazil	Brazil	Delicioso	Panamint	PWMF
Chunlei	China	China	Sunago Wase	Baixianglu	PWMC
Colibri	Brazil	Brazil	Cristal	Cristal	PWMC
Conserva 672	Brazil	Brazil	Topazio	Conserva 334	PYNmC
Coral 2	Brazil	Brazil	Mutation of Coral		PWMS
Crimson Lady	California, USA	USA	Grand Diamond	(Springcrest x Springcrest Mutation)	PYNmC
Danmo	Texas, USA	China	Ruiguang2	Early Red2	NYMC
Delicioso	Brazil	Brazil	Unknown	Unknown	PWMF
Della Nona	Brazil	Brazil	(Delicioso x Nectared 5)	Unknown	PWMF
Denjiulo	China	China	Unknown	Unknown	PWMF
Diamante	Brazil	Brazil	Convenio	(Cardeal x Aldrighi)OP	PYNmC
Dixiland	Texas, USA	USA	FV556	Dixigem	PYMF

Table 2. Continued.

Cultivar Name	Geographic Source of Sample	Genetic Origin	Female Parent	Male Parent	Fruit Traits[z]
Early August Prince	Georgia, USA	USA	Sunprince	BY92P2710	PYMF
Eldorado	Brazil	Brazil	Guaderio	Serrano	PONmC
Elegant Lady	California, USA	USA	Early O'Henry	July Lady	PYMF
Empress	Texas, USA	USA	(Flory Dwarf x Red Grand)	Unknown	PYMC
Eragil	Brazil	Brazil	Unknown	Unknown	PYMF
Esmeralda	Brazil	Brazil	Alpes	RR37201	PONmC
Fancy Lady	California, USA	USA	Mutation of Sparkle		PYMF
Fay Elberta	California, USA	USA	Unknown	Unknown	PYMF
Fayette	California, USA	USA	Fay Elberta	FV8914	PYMF
Fengbai	China	China	Okubo	Unknown	PWMF
Fireprince	Georgia, USA	USA	FV61130	FV32425	PYMF
Flameprince	Texas, USA	USA	BY68-3877	Unknown	PYMF
Flordacrest	Texas, USA	USA	FLA5-13N	Flordaking	PYMC
Flordadawn	Texas, USA	USA	Flordagold	Earligrande	PYMC
Flordaglo	Brazil	USA	Sundowner	Maravilha	PWMC
FlordaGrande	Brazil	USA	FLA5-58	(Flordasun x Springtime)	PYMF

Table 2. Continued.

Cultivar Name	Geographic Source of Sample	Genetic Origin	Female Parent	Male Parent	Fruit Traits[z]
Flordaguard	Texas, USA	USA	FLA4-115	Unknown	PYMF
Flordaking	Texas, USA	USA	FLA9-67	Early Amber	PYMC
Flordaprince	Texas, USA	USA	FLA2-7	Maravilha	PYMC
FlordaRio	Texas, USA	USA	Harken	Earligrande	PYMC
Galaxy	Texas, USA	USA	P34-106	D33-1	PWMC
Gaúcho[y]	Brazil	Brazil	Unknown	Unknown	PWMS
Gaúcho Porto Alegre	Brazil	Brazil	Delicioso	Unknown	PWMS
Gaudeiro	Brazil	Brazil	Delicioso	Interludio	PYMC
Guapo	Brazil	Brazil	(Sunhigh x Redcrest)	Unknown	PYMF
Giant Babcock	California, USA	USA	Babcock	July Elberta	PWMF
Goldprince	Texas, USA	USA	Loring	FV3257	PYMS
Granada	Brazil	Brazil	Conserva 471	Unknown	PYMC
Granito	Brazil	Brazil	Alpes	Conserva 102	PONmC
Gulfking	Texas, USA	USA	BY87P285	UFGold	PYNmC
Gulfprince	Texas, USA	USA	Aztec Gold	OroA	PYNmC
Hakuho	China	Japan	Baitao	Tachibana Wase	PWMC
Harvester	Texas, USA	USA	Redskin	Southern Glow	PYMF
Hongchizhi	Texas, USA	China	Unknown	Unknown	PW--
Huangnianhe	Texas, USA	China	Ruiguang 3	Armking	PYMC

Table 2. Continued.

Cultivar Name	Geographic Source of Sample	Genetic Origin	Female Parent	Male Parent	Fruit Traits[z]
Interludio	Brazil	Brazil	(Southland x Jewel)F_1	Unknown	PYMF
Jade	Brazil	Brazil	(Alpes x RR53272)	Unknown	PYNmC
Josefina	Brazil	Brazil	(Ouromel x Sunred)F_2	Unknown	NWMF
Julyprince	Georgia, USA	USA	L75-A50-20	BY89P2787	PYMF
June Gold	Texas, USA	USA	Flamingo	Springtime	PYMS
Juneprince	Texas, USA	USA	FV32558	June Gold	PYMS
Khun Wang	Thailand	Thailand	Unknown	Unknown	PWMF
Kurakto Wase	China	Japan	(Tashengan x Baitao)	Unknown	PWNmC
La Feliciana	Texas, USA	USA	L5-20-18	Unknown	PYMF
Laçador	Brazil	Brazil	Belvedere	Unknown	PYMF
Leonense	Brazil	Brazil	(Brilhante x NJC97)	Unknown	PYNmC
Long124	California, USA	China	Unknown	Unknown	PYMC
Maciel	Brazil	Brazil	Conserva 171	Conserva 334	PYNmC
Madrugador	Brazil	Brazil	(Aldrighi x Taquari Precoce)	Unknown	PYNmC
Marli	Brazil	Brazil	Delicioso	Preludio	PWMS
Natal	Brazil	Brazil	Suber	Toschina	PWMC
NJC137	Texas, USA	USA x Brazil	NJC83	Conserva 485	PONmC

Table 2. Continued.

Cultivar Name	Geographic Source of Sample	Genetic Origin	Female Parent	Male Parent	Fruit Traits[z]
O'Henry	California, USA	USA	Merrill Bonanza	Unknown	PYMF
Okinawa	Brazil	Japan	Unknown	Unknown	PWMF
Okubo	China	Japan	(Baitao x Unknown)	Unknown	PWMF
Olimpia	Brazil	Brazil	Bolinha	7-28	PYNmC
Perola de Itaquera	Brazil	Brazil	Unknown	Unknown	PWNmC
Pilcha	Brazil	Brazil	Precoce Rosado	Unknown	PYMC
Pingbaizi	California, USA	China	Unknown	Unknown	PWMF
Red Angkhang	Thailand	Thailand	Unknown	Unknown	PWMF
Redhaven	Texas, USA	USA	Halehaven	Kalhaven	PYMC
Regal	Texas, USA	USA	Harvester	Surecrop	PYMC
Rich Lady	Texas, USA	USA	Amparo	Unknown	PYMS
Riograndense	Brazil	Brazil	(Brilhante x NJC97)	Unknown	PYNmC
Rosalia	Brazil	Brazil	Unknown	Unknown	NYNC
Rubyprince	Georgia, USA	USA	Fireprince	BY78GN55	PYMC
Ruston Red	Texas, USA	USA	La Premiere	L63-2-29	PYNmC
Saavedra	Brazil	Bolivia	Unknown	Unknown	PWNC
Santa Áurea	Brazil	Brazil	Cerrito	NJC88	PYNmC
Scarlet Prince	Georgia, USA	USA	Blaze Prince	Unknown	PYMC

Table 2. Continued.

Cultivar Name	Geographic Source of Sample	Genetic Origin	Female Parent	Male Parent	Fruit Traits[z]
Schatea-F	California, USA	France	Sanguine de Chateau Neuf	Unknown	PRM-
Serodio	Brazil	Brazil	(Admirável x Delicioso)	Unknown	PYMC
Sinuelo	Brazil	Brazil	Preludio	Amarelinho	PWMF
Southern Pearl	Texas, USA	USA	Rose Princess	Unknown	PWMS
Springold	Texas, USA	USA	FV8914	Springtime	PYMC
SpringHoney	California, USA	Taiwan	Premier	Flordabelle	PWMC
Springprince	Texas, USA	USA	Springcrest	Unknown	PYNmC
Stard-A	Texas, USA	France	Sanguine Tardif de Chanas	Unknown	PRMF
Stard-G	Texas, USA	France	Sanguine Tardif de Chanas	Unknown	PRMF
Stard-R	Texas, USA	France	Sanguine Tardif de Chanas	Unknown	PRMF
Summerprince	Texas, USA	USA	BY8-3877	Unknown	PYMS
Summerset	California, USA	USA	Kirkman Gem	(J H Hale x Rio Oso Gem)	PYMF
Sunago Wase	China	Japan	Unknown	Unknown	PWMC
Sunblaze	Brazil	USA	FLA3-4N	FLA5-9	PYMS
Suncoast	Texas, USA	USA	FLA9-12N	FLA7-3N	NYMC

Table 2. Continued.

Cultivar Name	Geographic Source of Sample	Genetic Origin	Female Parent	Male Parent	Fruit Traits[z]
Sunfre	Texas, USA	USA	P4281	P4291	NYMC
Sunmist	Brazil	USA	Flordaglo	Mayfire	NWMC
Sunraycer	Texas, USA	USA	FLA7-11	FLA7-3N	NYMC
Sureprince	Texas, USA	USA	(Dixiland x Durbin)	Unknown	PYMS
Taichi	Brazil	Brazil	Unknown	Unknown	PWMF
Taiwan1	Thailand	Taiwan	Ingetaur	Unknown	PW--
Taiwan2	Thailand	Taiwan	Kuutaur	Unknown	PW--
Talisma	Brazil	Brazil	Rei da Conserva	Jewel	PWMC
TexKing	Texas, USA	USA	Goldprince	TX3290-2	PYMC
TexPrince	Texas, USA	USA	P60-12	Flordaking	PYMF
TexRoyal	Texas, USA	USA	NJ239	Early Amber	PYMF
Texstar	Brazil	USA	Unknown	Unknown	PYMC
TropicBeauty	Texas, USA	USA	FLA3-2	Flordaprince	PYMC
TropicPrince	Texas, USA	USA	TropicBeauty	TropicBeauty	PYMC
TropicSnow	Brazil	USA	FLA7-11	Maravilha	PWMS
TropicSweet	Brazil	USA	FLA4695	Kaygold	PYMS
Turmalina	Brazil	Brazil	Conserva 334	Conserva 594	PYNmC
Tutu	Brazil	Brazil	Rei da Conserva	Jewel	PWMF
TX1A95	Texas, USA	USA	TXW1193-1	Unknown	PYMC
TX2A232LWN	Texas, USA	USA	Sunmist	Arctic Star	NWMC

Table 2. Continued.

Cultivar Name	Geographic Source of Sample	Genetic Origin	Female Parent	Male Parent	Fruit Traits[z]
TX3B195N	Texas, USA	USA	Crimson Baby	A402CN	NYMC
TX3E213LW	Texas, USA	USA	TX4D46W	Summer Sweet	PWMS
TX4D208W	Texas, USA	USA x China	TXW1591-1	Zhaohuangzhu	PWMC
TX4F194LW	Texas, USA	USA x China	TXW1591-1	Zhaohuangzhu	PWMC
TX4F223LW	Texas, USA	USA x China	TXW1591-1	Zhaohuangzhu	PWMC
TXW1A20	Texas, USA	USA	TXW1392-1	Unknown	PYMC
Uvilla	Brazil	Bolivia	Unknown	Unknown	PWMF
Vanguarda	Brazil	Brazil	(Alpes x RR53272)	Unknown	PYNmC
Victor	Texas, USA	USA	Tropic Beauty	Goldprince	PYMC
White Angkhang	Thailand	Thailand	Unknown	Unknown	PWMF
White Robin	Texas, USA	USA	FLA21-74	FLA3-71	PWMC
Xiantao	California, USA	China	Unknown	Unknown	PWMC
Xijao #1	California, USA	China	Unknown	Unknown	PYNmC
Yanguang	Texas, USA	China	Ruiguang3	Arm King	NWMC
Ying Ku	Thailand	Thailand	Unknown	Unknown	PW-F
Yuhualu	China	China	Baihua	Early Chinese Cling	PWMS

Table 2. Continued.

Cultivar Name	Geographic Source of Sample	Genetic Origin	Female Parent	Male Parent	Fruit Traits[z]
Zaolupanto	China	China	Sahuahongpanto	Zaoxiangyu	PWMC

[z]Fruit traits are as follows: First letter fruit type, P = Peach, N = Nectarine; Second letter flesh color Y = Yellow, W = White, O = Orange; Third letter M = Melting flesh, Nm = Non-melting flesh; Fourth letter clingstone type C = Clingstone, S = Semi, F = Freestone.

[y]Although the exact parentage of 'Gaúcho' is unknown, it has been reported by Maria Raseira of EMBRAPA to have 'Delicioso' in its background.

DNA Extraction

The DNA extraction procedure was a modification of the Doyle and Doyle (1987) method as described below. All chemicals used in this extraction process are listed in Appendix A. The procedure was performed by weighing out approximately 50 mg of young, unexpanded leaf tissue and placing it in a 1.5 mL microcentrifuge tube. Liquid nitrogen was poured into and around the microcentrifuge tube and the tissue crushed with a microcentrifuge pestle attached to a cordless drill. After the tissue was thoroughly crushed, 700 µL of 2x CTAB was added and tubes were vortexed vigorously for approximately 10 seconds. After vortexing, tubes were placed in a water bath (65°C) for 2.5 hours. Samples were then removed from the water bath and allowed to come to room temperature. Seven hundred µL of CIA was added to each tube and inverted several times to assure mixing. Centrifugation of samples occurred at 13,200 g_n for 10 minutes and was repeated if the top layer was not clear and colorless. The top aqueous layer was removed and placed in a new 1.5 mL microcentrifuge tube and 700 µL of CIA was added to new tube. Samples were inverted several times and then placed on the vortex briefly to insure thorough mixing. Once again, centrifugation occurred at 13,200 g_n for 10 minutes and repeated if top layer was not clear and colorless. The top aqueous layer was transferred to a new microcentrifuge tube and 500 µL of cold (-20ºC) isopropanol was added to each new microcentrifuge tube. After addition of cold isopropanol, tubes were inverted several times and DNA precipitation was usually visible during this step. Tubes were placed in the freezer (-20ºC) overnight followed by centrifugation at 6000 g_n for 10 minutes. The supernatant was carefully poured out and

the tubes were inverted on a paper towel to dry. The DNA pellet was washed with 70% ethanol two times. To help lodge the DNA pellet back down into the tube, it was centrifuged for approximately one minute. The ethanol was carefully poured off and the tube was allowed to air dry at room temperature. Once tube was dry, 50 µL of TE was added to each tube and vortexed for 10 minutes. Samples were stored at -20°C.

DNA Quantification

The extracted DNA was quantified by the use of a Hoefer DQ 300 fluorometer. Once the stock DNA concentration was determined, a working stock was created by diluting all DNAs to 5 $ng \cdot \mu L^{-1}$ using nuclease free water.

PCR Amplification

Polymerase Chain Reactions (PCR) were performed in a total volume of 10 µL containing 5 µL of Phusion Flash High-Fidelity PCR Master Mix (New England BioLabs, Inc.), 3 µL of Nuclease Free Water, 0.5 µL of each forward and reverse primers (2.5 pmol/µL stock) and 1 µL of DNA (5 ng/µL). Annealing temperature for each primer pair was optimized by adapting published information and differed for each primer pair used (Table 2). PCR cycling was performed on a Techne TC-412 Thermal Cycler (Barloworld Scientific, Ltd.) under the following conditions: 105°C heated lid, 10 s initial denaturation at 98ºC, 30 cycles of amplification (1 s at 55°C, 5 s at annealing temperature, 1 min at 72ºC) followed by a final extension of 1 min at 72°C. PCR product was confirmed on a 3% MetaPhor agarose gel.

Table 3. SSR primers utilized.

Primer Name	Fluorescent Label	Annealing Temperature	Reference	No. of Alleles	Size Range in bp	PIC	H_o	H_e
BPPCT007	HEX	52	Dirlewanger *et al.* , 2002	6	125-147	0.60	0.34	0.66
BPPCT014	FAM	57	Dirlewanger *et al.* , 2002	7	161-224	0.53	0.45	0.59
BPPCT025	HEX	57	Dirlewanger *et al.* , 2002	9	174-196	0.72	0.49	0.74
BPPCT028	FAM	57	Dirlewanger *et al.* , 2002	5	152-167	0.67	0.34	0.72
BPPCT034	HEX	58	Dirlewanger *et al.* , 2002	6	212-238	0.42	0.13	0.46
M12a	FAM	55	Yamamoto *et al.* , 2002	7	141-156	0.72	0.55	0.76
MA27	HEX	55	Yamamoto *et al.* , 2002	4	173-197	0.49	0.12	0.57
Pchgms1	HEX	57	Sosinski *et al.* , 2000	5	191-204	0.30	0.26	0.32
Pchgms3	FAM	55	Sosinski *et al.* , 2000	7	166-208	0.46	0.25	0.50
UDP96-005	FAM	57	Cipriani *et al.* , 1998	9	145-172	0.70	0.45	0.74
UDP97-401	HEX	57	Cipriani *et al.* , 1998	6	117-141	0.49	0.32	0.58
Average				6.5		0.55	0.34	0.60

SSR Screening

A total of 77 SSR primer pairs were initially screened using 6 DNA samples from diverse geographical locations. Primer pairs were selected based on amplification ability, ease of scoring, polymorphic nature, commonality among labs and suggestions from the National Clonal Germplasm Repository for Fruit and Nut Crops. Eleven primers that met the above criteria were selected, used with all DNA selections and analyzed via capillary electrophoresis (Table 3).

Capillary Electrophoresis

Selected primers had the 5' end of the forward primer labeled with either FAM or HEX fluorescent dye (Table 3). PCR products of the FAM labeled primers were too strong to be analyzed directly and had to be diluted 50-100x prior to use. One µL of diluted FAM product and 1 µL of HEX product were mixed together in 8 µL of a Hi-Di formamide solution containing an internal size standard (850 µL of Hi-Di formamide

plus 50 μL of Genescan 400HD [Rox] both from Applied Biosystem, Inc.) Care was taken to ensure that no overlap occurred between the allele sizes of the FAM and HEX PCR products. Denaturation of products occurred on an ABI GeneAmp PCR System 2700 (Applied Biosystems, Inc.) immediately before analysis. Electrophoresis analysis was performed on an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, Inc.).

Data Analysis

To help assess the usefulness of the chosen primers, observed heterozygosity (H_o), expected heterozygosity (H_e) and polymorphism information content (PIC) values were calculated using CERVUS v. 3.0 (Kalinowski et al., 2007).

Peaks generated by the ABI 3100 Genetic Analyzer were analyzed with Peak Scanner software v. 1.0 (Applied Biosystems, Inc.). SSR peak analysis was scored as present (1) or absent (0) for each primer and recorded in a matrix form. This matrix was analyzed by NTSYSpc v. 2.2 (Numerical Taxonomy and Multivariate Analysis System; Rohlf, 2008) by using Nei and Li's Dice similarity coefficient (Nei and Li, 1979) to form a similarity matrix. Furthermore, NTSYSpc analyzed the similarity matrix with UPGMA (Unweighted Pair-Group Method, Arithmetic Mean) to give cluster analysis data and construct a dendrogram.

RESULTS AND DISCUSSION

Polymorphism

The total number of polymorphic bands generated by the selected primers was 72. The number of banding patterns combined with the number of primers used translates into an average number of 6.5 alleles per primer with the lowest being 4 (MA27) and the highest being 9 (BPPCT025 and UDP96-005). The average number of alleles found is higher than many other *Prunus persica* studies (Table 4). This can be explained by several factors such as the breadth of the germplasm surveyed, the number of cultivars studied, the number of primers used and even the methods used to detect alleles (refer to Table 4 for references). However, the alleles per locus found in this study is lower than that conducted by Yoon et al. (2006) on Chinese peaches in which 96 cultivars produced 283 alleles for an average of 8.6 alleles per locus. Even though a smaller number of cultivars were studied by Yoon et al. (2006) as compared to the present study, the Yoon study examined a more diverse selection of cultivars including ornamental peaches which are distinct from the fruiting types (Hu et al., 2005).

The average number of alleles found in peach as compared with those of other *Prunus* species (such as Almonds and Apricots) and other Rosaceae family members is lower (Table 4). This is due to the self fertile nature of peaches as compared to the outcrossing systems such as those found in almonds, apples, plums and sweet cherries (Byrne, 1990; Wiersma et al., 2001).

Table 4. Comparison of SSR allele diversity among various Rosaceae family members.

Common Name	Genus species	No. of Cultivars Analyzed	No. of SSR Primers Used	Total No. of Alleles	Average No. of Alleles per Primer	Citation
Peach	*Prunus persica*	15	21	52	2.5	Baranek et al., 2006
Peach	*Prunus persica*	28	10	26	2.6	Sosinski et al., 2000
Peach	*Prunus persica*	85	10	35	3.5	Wünsch et al., 2006
Peach	*Prunus persica*	50	26	118	4.5	Testolin et al., 2000
Peach	*Prunus persica*	100	7	32	4.6	Aranaza et al., 2001
Peach	*Prunus persica*	96	33	283	8.6	Yoon et al., 2006
Almond	*Prunus communis*	36	7	52	7.4	Xu et al., 2004
Apricot	*Prunus armeniaca*	25	21	141	6.4	Lopes et al., 2002
Apricot	*Prunus armeniaca*	74	12	107	7.6	Zhebentyayeva et al., 2003
Apricot	*Prunus armeniaca*	136	10	133	13.3	Maghuly et al., 2005
Rose	*Rosa chinensis*	90	23	291	12.7	Soules, 2009
Rose	*Rosa hybrida*	76	24	260	11	Esselink et al., 2003
Apple	*Malus x domestica*	66	8	97	12.1	Hokanson et al., 1998
Apple	*Malus x domestica*	41	13	84	6.5	Goulão and Oliveira, 2001

Polymorphism information content (PIC) values show the variability for each locus tested. A wide variation from 0.30 (Pchgms1) to 0.72 (BPPCT025 and M12a) with the average value being 0.56 was observed in this study (Table 3). Once again, the average PIC value determined for this study was found to be lower than the one found by Yoon et al. (2006). Unfortunately, no other PIC value reports could be found for *Prunus persica*. Studies on other *Prunus* species, such as one conducted on 36 almond cultivars (*Prunus communis* Fritsch.) by Xu et al. (2004) found a total of 18 primers gave an average PIC value of 0.631 with a range from 0.131 to 0.865.

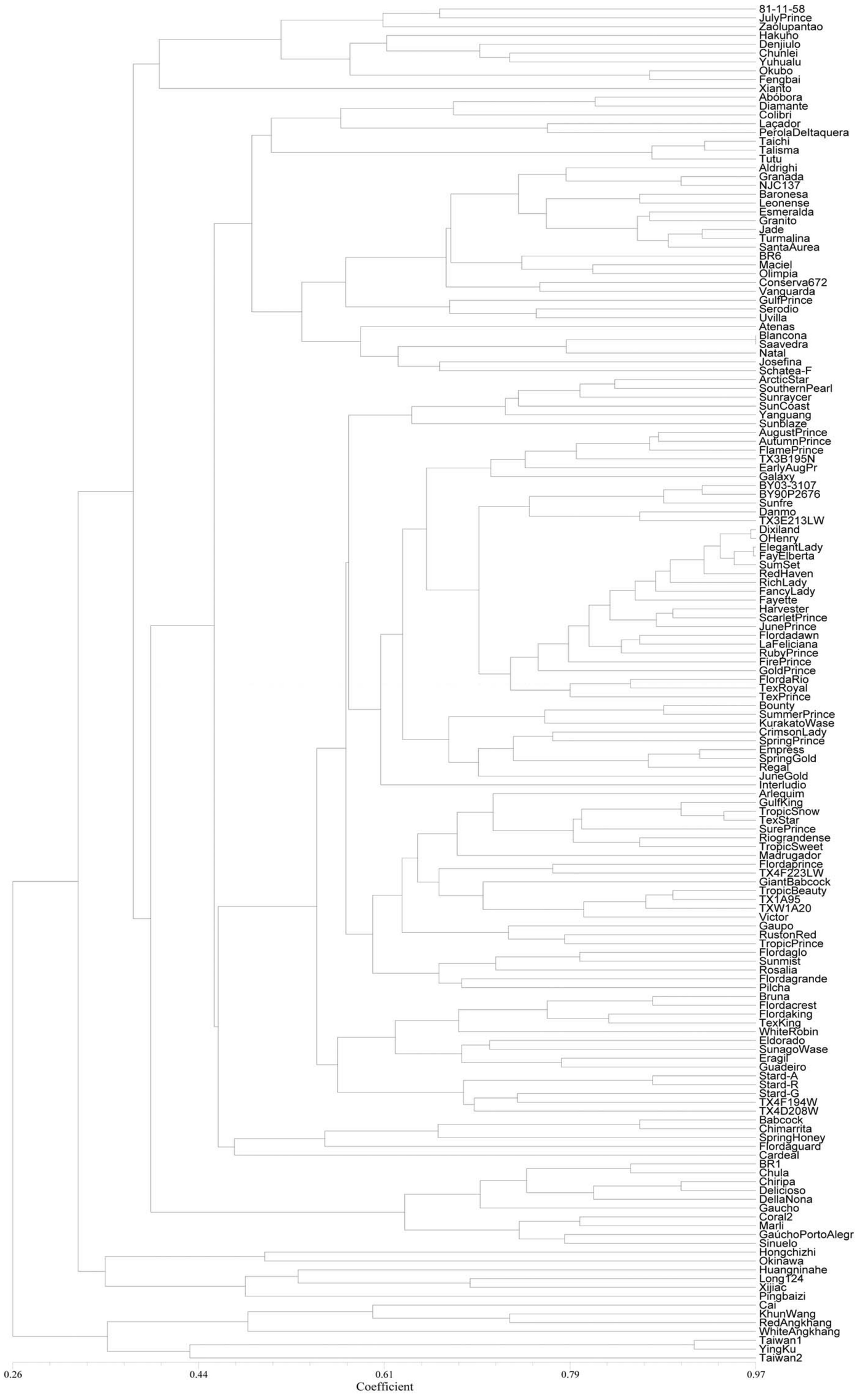
The average observed heterozygosity for the primers in this study was found to be 0.34 with a range from 0.12 (MA27) to 0.49 (BPPCT025) (Table 3). Expected heterozygosity ranged from 0.32 (Pchgms1) to 0.76 (M12a) with an average of 0.60 (Table 3). This is higher than the mean expected heterozygosity found by Dirlewanger et al. (2002) and Aranzana et al. (2002) which was 0.41 for both studies. In all cases, expected heterozygosity was greater than the observed heterozygosity. Low heterozygosity values generally indicate a reduced level of genetic variation due to repeated inbreeding and are most probably due to the self fertile nature of the peach.

SSR Dendrogram Results

The dendrogram shows seven distinct groups (with further subdivisions) of peaches among the genotypes tested (Figure 2). Group one shows a clustering of improved type peaches from Japan and the eastern regions of China. In general this group consists of high chill (650+ chill hours), white fleshed, melting type peaches with the exception of cultivars 'Fengbai' and 'Okubo' which are clingstone. Similarity coefficients range from 0.51 to 0.87 for this group (Figure 2). This finding is similar to that of Yoon et al. (2006), in which the Japanese peach cultivars studied grouped closely to the southern Chinese local and North American cultivars.

Group two shows a clustering of peaches that are from Brazil which can be further subdivided into two additional groups. Group 2A contains three important founding clones of Brazilian peaches: 'Abóbora', 'Perola De Itaquera' and 'Taichi'. Furthermore, this group indirectly contains a fourth founding clone, 'Rei da Conserva'

Figure 2. Dendrogram of *Prunus persica* cultivars/genotypes. The UPGMA (Unweighted Pair-Group Method, Arithmetic Mean) clustering method was used to construct a dendrogram based on Nei and Li's Dice similarity coefficients obtained from SSR results.


81-11-58
JulyPrince
Zaolupantao
Hakuho
Denjiulo
Chunlei
Yuhualu
Okubo
Fengbai
Xianto
Abóbora
Diamante
Colibri
Laçador
PerolaDeItaquera
Taichi
Talisma
Tutu
Aldrighi
Granada
NJC137
Baronesa
Leonense
Esmeralda
Granito
Jade
Turmalina
SantaAurea
BR6
Maciel
Olimpia
Conserva672
Vanguarda
GulfPrince
Serodio
Uvilla
Atenas
Blancona
Saavedra
Natal
Josefina
Schatea-F
ArcticStar
SouthernPearl
Sunraycer
SunCoast
Yanguang
Sunblaze
AugustPrince
AutumnPrince
FlamePrince
TX3B195N
EarlyAugPr
Galaxy
BY03-3107
BY90P2676
Sunfre
Danmo
TX3E213LW
Dixiland
OHenry
ElegantLady
FayElberta
SumSet
RedHaven
RichLady
FancyLady
Fayette
Harvester
ScarletPrince
JunePrince
Flordadawn
LaFeliciana
RubyPrince
FirePrince
GoldPrince
FlordaRio
TexRoyal
TexPrince
Bounty
SummerPrince
KurakatoWase
CrimsonLady
SpringPrince
Empress
SpringGold
Regal
JuneGold
Interludio
Arlequim
GulfKing
TropicSnow
TexStar
SurePrince
Riograndense
TropicSweet
Madrugador
Floridaprince
TX4F223LW
GiantBabcock
TropicBeauty
TX1A95
TXW1A20
Victor
Gaupo
RustonRed
TropicPrince
Flordaglo
Sunmist
Rosalia
Flordagrande
Pilcha
Bruna
Flordacrest
Flordaking
TexKing
WhiteRobin
Eldorado
SunagoWase
Eragil
Guadeiro
Stard-A
Stard-R
Stard-G
TX4F194W
TX4D208W
Babcock
Chimarrita
SpringHoney
Floridaguard
Cardeal
BR1
Chula
Chiripa
Delicioso
DellaNona
Gaucho
Coral2
Marli
GaúchoPortoAlegr
Sinuelo
Hongchizhi
Okinawa
Huangninahe
Long124
Xijiao
Pingbaizi
Cai
KhunWang
RedAngkhang
WhiteAngkhang
Taiwan1
YingKu
Taiwan2
0.26
0.44
0.61
0.79
0.97
Coefficient

as the female parent of ‘Talisma’ and ‘Tutu’. The three founding clones (‘Perola De Itaquera’, ‘Rei da Conserva’ and ‘Taichi’) were used by the south-central Brazil peach breeding program of São Paulo principally for the development of melting type peaches for the fresh market. Whereas ‘Abóbora’ was used in the breeding of non-melting cultivars in the program in Pelotas.

Group 2B consists mainly of non-melting peaches from Brazil and contains the Brazilian founding clone ‘Aldrighi’ and indirectly the founding clones of ‘Ambrósio Perret’, ‘Lake City’ and ‘Suber’. The three founding clones of ‘Aldrighi’, ‘Ambrósio Perret’ and ‘Lake City’ were used by the southernmost Brazilian peach breeding program at Pelotas for the development of non-melting peaches suitable for the processing industry. There are two selections from the USA, ‘Gulfprince’ and NJC137, and three selections from Bolivia, ‘Blancona’, ‘Saavedra’ and ‘Uvilla’ that cluster within this group as well. ‘Gulfprince’ has the founding clones of ‘Mexican Cling’ and ‘Abóbora’ in its background and is a non-melting type peach. Upon viewing the parentage of NJC137 it was determined that ‘Conserva 485’ is the pollen parent. ‘Conserva 485’ is a peach developed in Brazil with a parentage of ‘Alpes’ x ‘Conserva 102’. Furthermore, ‘Conserva 102’ has the founding clone of ‘Aldrighi’ as its female parent. One peach from France, Schatea-F (‘Sanguine de Chateau Neuf’ selection), a traditional high chill, late ripening, red flesh peach clusters in this group as well.

The largest number of peaches cluster within group 3A (Figure 2). This group contains a mixture of peaches and nectarines that are mainly yellow fleshed, melting

with a wide range of chilling requirements and fruit ripening periods. With few exceptions, the majority of this group was bred and released in the USA.

Two peaches, ‘Yanguang’ and ‘Danmo’ which were developed in China, cluster in group 3A due to their complex USA/Chinese parentage. A few Brazilian peaches, ‘Interludio’ and ‘Guapo’, cluster within this group as well. Even though ‘Interludio’ and ‘Guapo’ are both peaches used in Brazil, they are selections with parents of USA origin. ‘Bruna’ is a nectarine that arose out of a selection of seeds that were developed by Rutgers University and shipped to Brazil. The background of the Brazilian nectarine ‘Rosalia’ is currently unknown, however it has a yellow flesh with a melting texture. Very little is known about the Brazilian peach ‘Pilcha’. The most immediate female parent of ‘Pilcha’ is the low chill Brazilian founding clone of ‘Precoce Rosado’ which may suggest that this founding clone is very similar to the melting type germplasm used in the USA. One other Brazilian peach, ‘Riograndense’, clusters within this group as well. Although nothing is known about the pollen parent of this peach, the female parent is listed as a complex Brazilian-USA hybrid and could account for its position within this dendrogram.

Two Japanese peaches cluster here as well, ‘Kurakato Wase’ and ‘Sunago Wase’. ‘Kurakato Wase’ is a peach that was created in Japan using a USA peach (‘Tuscan’) as its female and a Chinese peach as the male parent. The parentage of ‘Sunago Wase’ is unknown and is reported as a probable ‘Okubo’ seedling (Wang, personal communication). The Japanese peach ‘Okubo’ has been analyzed in this study and found to cluster in group one. It is, therefore, unlikely that ‘Sunago Wase’ is a seedling

of ‘Okubo’ based on this study. The majority of the French red fleshed peaches cluster within this group as well. Although Stard-A, Stard-G and Stard-R (‘Sanguine Tardif de Chanas’ selections) are siblings, they are not identical as can be seen in the dendrogram.

Groups 3B, 3C and 4 consist mainly of melting flesh type peaches from Brazil and the United States of America. Looking at the relationships in 3B, we find that the female parent of ‘Chimarrita’ is ‘Babcock’ (Figure 2). ‘Spring Honey’ and ‘Chimarrita’ share the same male parent (‘Flordabelle’). The most distantly related accessions in group three are ‘Flordaguard’ (3B) and ‘Sunblaze’ (3C). ‘Flordaguard’ is a rootstock that was bred and released for its nematode resistance qualities and therefore it is not surprising that it groups distantly (0.48 similarity) from group 3A.

Group four contains only the cultivar ‘Cardeal’ which is derived from a ‘Southland’ x ‘Hawaiian’ population. This population was used in the initial University of Florida peach breeding program for its low chill and melting flesh characteristics (Byrne and Bacon, 1999).

Group five contains white fleshed, melting, freestone Brazilian peaches. All peaches in this group can be traced to the Brazilian founding clone of ‘Delicioso’. ‘Delicioso’ is the most important founding clone in the fresh market, melting flesh peach breeding program at Pelotas, Rio Grande do Sul, Brazil by contributing to the background of every cultivar release from 1976 up through 1999 (Byrne and Bacon, 1999).

Group six consists of two subgroups. Group 6A consists of the high chill, ornamental weeping cultivar ‘Hongchizhi’ (actual name ‘Honghuachongbanzhuizhitao’)

and the low chill rootstock ‘Okinawa’ originally from southern China. ‘Hongchizhi’ (from the Beijing municipality of China) and other ornamentals have been shown in a previous report to be distinct genetically from other cultivated peaches (Yoon et al., 2006). Although the peach rootstock ‘Okinawa’ was named in Japan it was collected from southern China, and has also been shown by a previous study to be distinct from other Chinese and US peach germplasm (Warburton and Bliss, 1996). The University of Florida used ‘Okinawa’ as a source for the low chill characteristic in its early breeding program (Byrne and Bacon 1999).

Group 6B contains Chinese cultivars from the northern regions of China with the exception of ‘Huangninahe’ which is from the Yunnan province located in southern China. The local Chinese cultivars of ‘Long124’ and ‘Xijiao#1’ are from the provinces of Gansu and Shannxi, respectively and ‘Pingbaizi’ is from the municipality of Nanjing. The similarity coefficient for the subgroups of 6A and 6B range from 0.35 to 0.70, and when group six as a whole is compared to the peaches found in group one the similarity coefficient is 0.31 (Figure 3). These findings are in general agreement with those found by Yoon, et al. (2006) and Promchot et al. (2005) in which northern Chinese peach types grouped separately from the southern Chinese/North American peach types.

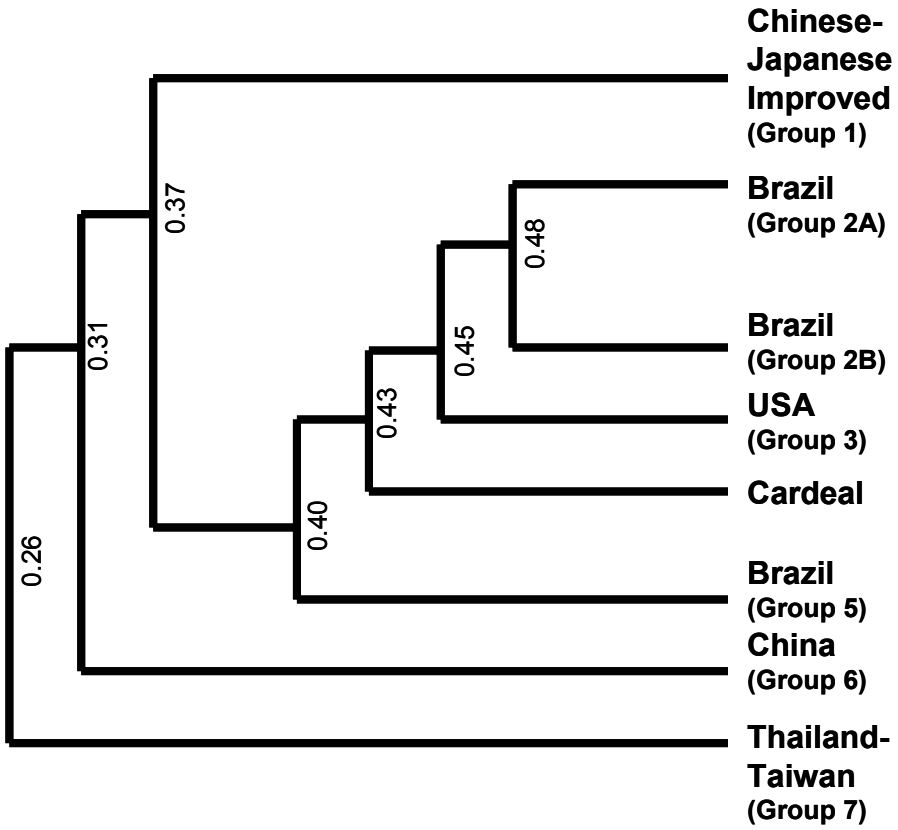


Figure 3. Dendrogram of the eight main groups. Numbers along vertical axis represent similarity coefficients.

The final group, seven, contains a cluster of low chill, naturalized peaches from Thailand and Taiwan with the notable exceptions of 'Xiantao' which is a high chill, ornamental Chinese peach and 'Cai' which is a Brazilian peach. The low chill germplasm from Taiwan and Thailand is the most distinct cluster of peaches in this study with a similarity coefficient of 0.26 (Figure 3). In addition, many of the individual accessions are also distantly related (similarity coefficient of 0.60 or lower) indicating a high degree of polymorphism in this peach group. This is in contrast to a study in Thailand that indicated that the Thai cultivars of 'Khun Wang', 'Red Angkhang' and

‘White Angkhang’ were closely related (> 90% similarity) to each other as well as to ‘TropicBeauty’ and ‘Okinawa’ (Promchot et al., 2005).

SUMMARY

Although not as diverse as other naturally out-crossing crops, peach does exhibit a wide range of diversity (Figure 2, Figure3). The distinct clustering of Chinese peaches (groups one and six), low chill Brazilian peaches (groups two, four and five), low chill Asian peaches (group seven) and USA/European peaches (group three) show varying degrees of relatedness, from 0.97 similarity between the Bolivian peaches 'Blancona' and 'Saavedra' to 0.33 similarity within group seven (Figure 2, Figure3).

The Chinese germplasm is represented by three categories: local (unimproved) cultivars, ornamental cultivars and southern China/Japanese cultivars. The discrete clustering habits of the Chinese peaches used in this study mirror other studies in which Chinese germplasm was distinct not only amongst itself (northern versus eastern and southern regions) but from other countries as well (Chen and Huang, 2009; Chen *et al*., 2007; Cheng, 2001; Gašić *et al*., 2000; Promchot *et al*., 2005; Warburton and Bliss, 1996; Wen and Chieh, 2003; Yoon *et al*., 2006).

Most interesting, perhaps, are the clustering tendencies of the Brazilian germplasm. The germplasm studied shows clear distinctions between the two low chill breeding programs of Pelotas and São Paulo with further distinctions within the Pelotas program of the melting and non-melting characteristic. The group that is represented in cluster 2A is the work of the São Paulo breeding program. Three of the four low chill founding clones ('Perola de Itaquera', 'Rei da Conserva' and 'Taichi') used in this group were exploited for the creation of fresh market, melting flesh peaches (Byrne and Bacon, 1999). A separate cluster of white fleshed, melting, freestone Brazilian peaches is

represented in group five, which contains the important founding clone ‘Delicioso’. This founding clone was utilized by the Pelotas breeding program to develop melting flesh peaches for the fresh market independently of the São Paulo program (Byrne and Bacon, 1999). Furthermore, the non-melting flesh, processing type peaches developed by the Pelotas breeding program are represented in group 2B by the founding clone of ‘Aldrighi’ and indirectly by ‘Ambrósio Perret’ and ‘Lake City’ as the female parent of BR6 and ‘Arlequim’ respectively.

The most distinct peaches found in this study are the low chill peaches from Asia. A wide range of diversity was observed, from the most similar cultivars ‘Taiwan1’ and ‘Ying Ku’ (0.91 similarity) to the group as a whole (0.33 similarity) (Figure 2, Figure3). This is in contrast to Promchot *et al*. (2005), who found that the local Thai peaches were very closely related (0.79 to 1). The Chinese cultivar ‘Xiantao’ clustered within the Thai group as well. ‘Xiantao’ is a high chill, ornamental peach that is from the southern Chinese province of Yunnan which is geographically close to Thailand. This suggests that peaches may have been brought to Thailand from China through the migration of people between the countries.

In this study, the largest group investigated was the USA peaches. Although this group subdivided into three distinct clusters, there was no clear distinction between the high, medium and low chill cultivars. This finding is supported by several other studies (see Table 1). However, there is a tendency for the low chill cultivars to cluster together (see ‘Flordaglo’, ‘Flordagrande’, ‘Pilcha’, ‘TropicPrince’, ‘TropicSnow’ and

‘TropicSweet’ through selection TXW1A20) but the important low chill founding clone derivative of ‘Flordaprince’ clusters in a group of medium chill cultivars (Figure 2).

Further work that needs to be undertaken includes the analysis of relationships among the low chill peach germplasm from Asia, Brazil, and the USA with that collected and developed in Mexico and to other parts of Central and South America. Additionally, the relationships among the non-melting germplasm, including native selections and improved cultivars from Asia, the USA, Central and South America and Europe, need to be investigated.

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APPENDIX A

CHEMICAL PREPARATIONS FOR DNA EXTRACTION

2X CTAB buffer (100 ml):

2% CTAB	2.00 g
1.4 M NaCl	8.12 g
20 mM EDTA, pH 8.0	4 ml of 0.5 M
100 mM Tris HCl, pH 8.0	10 ml of 1.0 M
1% PVP-40 (polyvinylpyrollidone, M.W. 40,000)	1.00 g
β-Mercaptoethanol	200 µL

Note: CTAB is difficult to dissolve. Do not add β-Mercaptoethanol until ready to use.

0.5M EDTA, pH 8 (1000 ml):

EDTA (Disodium ethylenediaminetetraacetate·$2H_2O$)	186.1 g

Preparation: Add 186.1 g of EDTA to 200 mL of water. Stir vigorously on a magnetic stirrer. Adjust the pH to 8 with NaOH (~20 g of NaOH pellets), then adjust volume of the solution to 1000 mL with water.

Note: EDTA will not go into solution until the pH of the solution is adjusted to approximately 8 by the addition of NaOH.

1.0 M Tris HCl, pH 8 (1000 ml):

Tris (Hydroxymethyl) Aminomethane	121.14 g

Preparation: Dissolve 121.14 g of Tris in 800 mL of water. Adjust the pH to 8 by adding HCl (~42 mL of concentrated HCl). Allow the solution to cool to room temperature before making final adjustment to the pH. Adjust volume of the solution to 1000 mL with water.

TE (100 mL):

10 mM Tris·HCl	1.0 mL of 1.0 M
1 mM EDTA	0.5 mL of 0.5 M

Note: Bring solution to 100 mL with nanopure water.

CIA (100 mL):

Chloroform	96 mL
Isoamyl Alcohol	4 mL

Note: Store CIA at -20°C.

APPENDIX B

LIST OF CULTIVARS/GENOTYPES WITH PLANT PATENTS AND CITATIONS

Cultivar Name	Plant Patent No.	Citation
81-11-58	N/A[z]	Wang, personal communication
Abóbora	N/A	Raseira, personal communication
Aldrighi	N/A	Raseira, personal communication
Arctic Star	9332	Okie, 1998
Arlequim	N/A	Topp et al., 2008
Atenas	N/A	Topp et al., 2008
Augustprince	N/A	Okie and Layne, 2008a
Autumnprince	N/A	Okie, 1999
Babcock	N/A	Okie, 1998
Baronesa	N/A	Raseira, personal communication
Blancona	N/A	Raseira, personal communication
Bounty	N/A	Okie, 1998
BR1	N/A	Raseira, personal communication
BR6	N/A	Topp et al., 2008
Bruna	N/A	Raseira, personal communication
BY03-3107s	N/A	Okie, personal communication
BY90P2676	N/A	Okie, personal communication
Cai	N/A	Raseira, personal communication

Appendix B Table. Continued.

Cultivar Name	Plant Patent No.	Citation
Cardeal	N/A	Raseira, personal communication
Chimarrita	N/A	Topp et al., 2008
Chiripa	N/A	Raseira, personal communication
Chula	N/A	Topp et al., 2008
Chunlei	N/A	Wang and Zhuan, 2001
Colibri	N/A	Raseira, personal communication
Conserva 672	N/A	Raseira, personal communication
Coral 2	N/A	Raseira, personal communication
Crimson Lady	7953	Bradford and Bradford, 1992
Danmo	N/A	Wang and Zhuan, 2001
Delicioso	N/A	Raseira, personal communication
Della Nona	N/A	Topp et al., 2008
Denijulo	N/A	Wang, personal communication
Diamante	N/A	Raseira, personal communication
Dixiland	N/A	Okie, 1998
Early Augustprince	N/A	Okie and Layne, 2008a
Eldorado	4780	Okie, 1998
Elegant Lady	4399	Okie, 1998
Empress	2533	Okie, 1998
Eragil	N/A	Raseira, personal communication
Esmeralda	N/A	Topp et al., 2008

Appendix B Table. Continued.

Cultivar Name	Plant Patent No.	Citation
Fancy Lady	7023	Mizuno amd Mizuno, 1989
Fay Elberta	N/A	Okie, 1998
Fayette	N/A	Okie, 1998
Fengbai	N/A	Wang and Zhuan, 2001
Fireprince	N/A	Okie, 1998
Flameprince	N/A	Okie, 1998
Flordacrest	N/A	Okie, 1998
Flordadawn	N/A	Okie, 1998
Flordaglo	N/A	Okie, 1998
FlordaGrande	N/A	Okie, 1998
Flordaguard	N/A	Okie, 1998
Flordaking	N/A	Okie, 1998
Flordaprince	N/A	Okie, 1998
FlordaRio	N/A	Okie, 1998
Galaxy	N/A	Ramming, 2005
Gaucho	N/A	Raseira, personal communication
Gaúcho Porto Alegre	N/A	Raseira, personal communication
Gaudeiro	N/A	Raseira, personal communication
Guapo	N/A	Raseira, personal communication
Giant Babcock	1353	Okie, 1998
Goldprince	N/A	Okie, 1998
Granada	N/A	Topp et al., 2008
Granito	N/A	Topp et al., 2008
Gulfking	14483	Sherman et al., 2004
Gulfprince	12686	Sherman et al., 2002

Appendix B Table. Continued.

Cultivar Name	Plant Patent No.	Citation
Hakuho	N/A	Wang, personal communication
Harvester	N/A	Okie, 1998
Hongchizhi	N/A	Wang, personal communication
Huangnianhe	N/A	Wang and Zhuan, 2001
Interludio	N/A	Raseira, personal communication
Jade	N/A	Topp et al., 2008
Josefina	N/A	Topp et al., 2008
Julyprince	N/A	Okie and Layne, 2008b
June Gold	1884	Okie, 1998
Juneprince	N/A	Okie, 1998
Khun Wang	N/A	Boonprakob, personal communication
Kurakto Wase	N/A	Wang, personal communication
La Feliciana	N/A	Okie, 1998
Laçador	N/A	Raseira, personal communication
Leonense	N/A	Topp et al., 2008
Long124	N/A	Wang and Zhuan, 2001
Maciel	N/A	Topp et al., 2008
Madrugador	N/A	Raseira, personal communication
Marli	N/A	Topp et al., 2008
Natal	N/A	Raseira, personal communication
NJC137	N/A	Goffreda, personal communication
O'Henry	2964	Okie, 1998

Appendix B Table. Continued.

Cultivar Name	Plant Patent No.	Citation
Okinawa	N/A	Okie, 1998
Okubo	N/A	Wang, personal communication
Olimpia	N/A	Topp et al., 2008
Perola de Itaquera	N/A	Raseira, personal communication
Pilcha	N/A	Topp et al., 2008
Pingbaizi	N/A	Wang and Zhuan, 2001
Red Angkhang	N/A	Boonprakob, personal communication
Redhaven	N/A	Brooks and Olmo, 1997
Regal	N/A	Okie, 1998
Rich Lady	N/A	Brooks and Olmo, 1997
Riograndense	N/A	Topp et al., 2008
Rosalia	N/A	Raseira, personal communication
Rubyprince	N/A	Okie, 1999
Ruston Red	N/A	Okie, 1998
Saavedra	N/A	Raseira, personal communication
Santa Áurea	N/A	Topp et al., 2008
Scarlet Prince	N/A	Okie and Layne, 2008b
Schatea-F	N/A	Byrne, personal communication
Serodio	N/A	Raseira, personal communication
Sinuelo	N/A	Raseira, personal communication
Southern Pearl	N/A	Okie, 1999
Springold	N/A	Okie, 1998
SpringHoney	N/A	Ou and Wen, 2003

Appendix B Table. Continued.

Cultivar Name	Plant Patent No.	Citation
Springprince	N/A	Okie, 1999
Stard-A	N/A	Byrne, personal communication
Stard-G	N/A	Byrne, personal communication
Stard-R	N/A	Byrne, personal communication
Summerprince	N/A	Okie, 1998
Summerset	N/A	Okie, 1998
Sunago Wase	N/A	Wang, personal communication
Sunblaze	N/A	Okie, 1998
Suncoast	N/A	Okie, 1998
Sunfre	N/A	Okie, 1998
Sunmist	N/A	Okie, 1998
Sunraycer	N/A	Okie, 1998
Sureprince	N/A	Okie, 1999
Taichi	N/A	Raseira, personal communication
Taiwan1	N/A	Boonprakob, personal communication
Taiwan2	N/A	Boonprakob, personal communication
Talisma	N/A	Raseira, personal communication
TexKing	14627	Byrne & Bacon, 2004b
TexPrince	14629	Byrne & Bacon, 2004a
TexRoyal	N/A	Okie, 1998
Texstar	N/A	Okie, 1998
TropicBeauty	N/A	Okie, 1998
TropicPrince	12965	Bryne, 2002b

Appendix B Table. Continued.

Cultivar Name	Plant Patent No.	Citation
TropicSnow	N/A	Okie, 1998
TropicSweet	N/A	Okie, 1998
Turmalina	N/A	Topp et al., 2008
Tutu	N/A	Raseira, personal communication
TX1A95	N/A	Byrne, personal Communication
TX2A232LWN	N/A	Byrne, personal Communication
TX3B195N	N/A	Byrne, personal Communication
TX3E213LW	N/A	Byrne, personal Communication
TX4D208W	N/A	Byrne, personal Communication
TX4F194LW	N/A	Byrne, personal Communication
TX4F223LW	N/A	Byrne, personal Communication
TXW1A20	N/A	Byrne, personal Communication
Uvilla	N/A	Raseira, personal communication
Vanguarda	N/A	Topp et al., 2008
Victor	N/A	Byrne, personal Communication
White Angkhang	N/A	Boonprakob, personal communication
White Robin	N/A	Beckman, et al., 2000
Xiantao	N/A	Wang, personal communication
Xijao #1	N/A	Wang and Zhuan, 2001

Appendix B Table. Continued.

Cultivar Name	Plant Patent No.	Citation
Yanguang	N/A	Wang, personal communication
Ying Ku	N/A	Boonprakob, personal communication
Yuhualu	N/A	Wang and Zhuan, 2001
Zaolupanto	N/A	Wang and Zhuan, 2001

[z]N/A = not applicable or not available.

VITA

Name: Natalie Ann Anderson

Address: Texas A&M University
Department of Horticultural Sciences
Mail Stop 2133
College Station, TX 77843-2133

Email Address: n-anderson@tamu.edu

Education: A.A.S, Biotechnology, North Harris Montgomery Community College, 1994
B.S., Biochemistry and Genetics, Texas A&M University, 1997
M.S., Horticulture, Texas A&M University, 2010